

Crossing the Mucosal Barrier: A Commensal Bacterium Gives Dengue Virus a Leg-Up in the Mosquito Midgut

Pascal Miesen¹, Ronald P. van Rij¹

¹ Department of Medical Microbiology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

* Correspondence: ronald.vanrij@radboudumc.nl

Commensal bacteria that colonize the midgut of *Aedes aegypti* mosquitoes can influence the transmission of arthropod-borne viruses. In this issue of *Cell Host & Microbe*, Wu et al. (2019) show that *Serratia marcescens* bacteria secrete enhancin proteins that cleave membrane-bound mucins, thereby facilitating dengue virus infection of midgut epithelial cells.

The mosquito *Aedes aegypti* is the most important vector for transmission of pathogenic arthropod-borne viruses (arboviruses) such as dengue virus (DENV), yellow-fever virus, Zika virus, and chikungunya virus. The capacity to transmit specific arboviruses by these mosquitoes (referred to as vector competence) is determined by a combination of factors that affect (1) the uptake of virus particles from an infectious blood meal, (2) replication in the midgut and secondary organs, and (3) shedding of infectious particles into the saliva. Vector competence is a dynamic trait that is defined by multiple variables including mosquito and virus genetics and antiviral immunity. In addition, it is becoming increasingly clear that the commensal gut microflora, consisting of dozens of bacterial and fungal species, has profound impact on virus replication, dissemination, and transmission (Dennison et al., 2014).

Infection of midgut epithelial cells is the first major tissue barrier to arbovirus transmission. After blood feeding, epithelial cells secrete a mixture of heavily glycosylated proteins (mucins) that form a thick mucus layer around the blood-clot in the midgut, the so-called peritrophic matrix, which bears some resemblance to the intestinal mucosal barrier of mammals. Interestingly, dissemination of DENV to secondary organs in the mosquito body is not affected by the formation of the peritrophic matrix, suggesting that this virus is able to infect midgut epithelial cells before the mucus layer is established (Kato et al., 2008).

Experimental manipulation of the microbiome has proven a powerful approach to study its effect on the susceptibility of mosquitoes to arbovirus infections. In this issue of *Cell Host & Microbe*, Wu et al. (2019) removed the gut microflora from *Aedes aegypti* by orally treating mosquitoes with antibiotics. Subsequent re-introduction of the commensal bacterium *Serratia marcescens*, but not 20 other commensals, increased DENV loads and prevalence, indicating that individual bacterial species differentially affect vector competence. Wu et al. (2019) identified a secreted bacterial protein, SmEnhancin, to be responsible for this effect. Blocking this protein or re-introducing *Serratia marcescens* Enhancin deletion mutants failed to boost DENV infectivity. Conversely, feeding the isolated recombinant protein increases susceptibility to DENV, indicating that SmEnhancin is required and sufficient to reinforce viral infection. Enhancin is a member of a family of microbial metalloproteases that target host glycoproteins and are likely important for colonization of vertebrate mucosa and the invertebrate gut. Enhancin was initially identified as a baculovirus-encoded protein that degrades intestinal mucins to enhance infection of the insect host (Wang and Granados, 1997).

Likewise, Wu et al. (2019) found that bacterial SmEnhancin facilitated DENV infection of midgut cells by cleaving off membrane-bound mucins.

The mechanisms by which specific members of the commensal gut microflora affect virus transmission are largely unknown, but some modes of action have been proposed (Figure 1) (Dennison et al., 2014). Indirectly, the microbiome can affect virus replication by priming immune cascades, mostly the NF- κ B-related Immune Deficiency (IMD) and Toll pathways (Barletta et al., 2017; Xi et al., 2008) or by competition for nutrients (Moreira et al., 2009). Direct effects of microbial products on vector competence have also been reported. A secreted *Talaromyces* fungal protein reduces the expression and activity of digestive enzymes, thereby delaying the inactivation of DENV viral particles in the midgut (Anglero-Rodriguez et al., 2017). In addition, some microbial products were proposed to directly target and degrade viral proteins as exemplified by the secretion of proteases by a chromobacterium species (Saraiva et al., 2018). The results by Wu et al. (2019) now expand the scope of mechanisms by showing that a bacterial enzyme directly weakens the mucus-dependent anatomical barrier that impedes infection of midgut epithelial cells.

It is well-established that mosquito populations differ in vector competence, which can be partly attributed to genetic polymorphisms, such as those in antiviral RNAi genes (Palmer et al., 2018). Wu et al. (2019) found that the presence of *Serratia marcescens* in wild-caught mosquito strains from different regions in China correlated well with increased virus replication, suggesting that the microbiota contributes to the variation in vector competence. Intriguingly, mosquitoes carrying *Serratia* were obtained from DENV endemic regions, whereas *Serratia*-negative mosquitoes were caught in non-DENV-endemic regions, which led the authors to propose that *S. marcescens* underlies the difference in DENV prevalence in these areas. Considering that *Serratia* species occur widespread in the environment and have a broad host tropism for various insect groups, it would be important to confirm this observation in *Aedes* mosquitoes from additional, more distant, arbovirus-endemic regions.

Another important insight from the study of Wu et al. (2019) concerns the role of mucins in arbovirus vector competence. Secreted mucins that constitute the peritrophic matrix are induced upon blood-feeding and thought not to affect DENV dissemination (Kato et al., 2008). In contrast, constitutive, non-blood-meal-induced mucins that are membrane-associated were identified by Wu et al. (2019) as dengue virus restriction factors. Interestingly, only SmEnhancin was able to cleave membrane-associated mucins, whereas enhancins from several other bacterial species specifically cleaved the secreted mucins. Furthermore, SmEnhancin enhanced infectivity of several arboviruses, DENV, Zika, and Sindbis virus in *Aedes aegypti*, but did not enhance Sindbis virus infection of *Culex pipiens* mosquitoes. These observations suggest that the cleavage activity of SmEnhancin shows remarkable specificity, the basis of which remains unknown. Given the widespread occurrence of *Serratia marcescens*, this is unlikely due to co-evolution of bacterium and mosquito host.

Future research should establish how broad virus restriction by membrane-bound mucins is across mosquitoes and other arboviral vectors, such as ticks and sandflies, and whether other bacteria target these constitutive mucins. These insights may lead to the development of new approaches to control local arbovirus transmission. A proof of concept that manipulation of endosymbiotic bacteria can interfere with virus transmission was shown by the introduction of the intracellular bacterium *Wolbachia* into *Aedes* mosquitoes, which reduced replication of multiple arboviruses. Release of *Wolbachia* infected mosquitoes is currently being tested as a transmission-blocking strategy in field trials in 12 countries. In this line of reasoning, Wu et al. (2019) propose that depletion of *Serratia marcescens* from *Aedes aegypti* midguts may serve as a new strategy to interfere with arbovirus

transmission. Yet, a putative bactericidal approach needs to specifically target *Serratia* species, as removing the entire gut microbiome renders mosquitoes more susceptible to arbovirus transmission (Xi et al., 2008). Nonetheless, further characterization of the mosquito microbiome and its interactions with arboviruses promises to open avenues for the development of novel strategies to block arbovirus transmission.

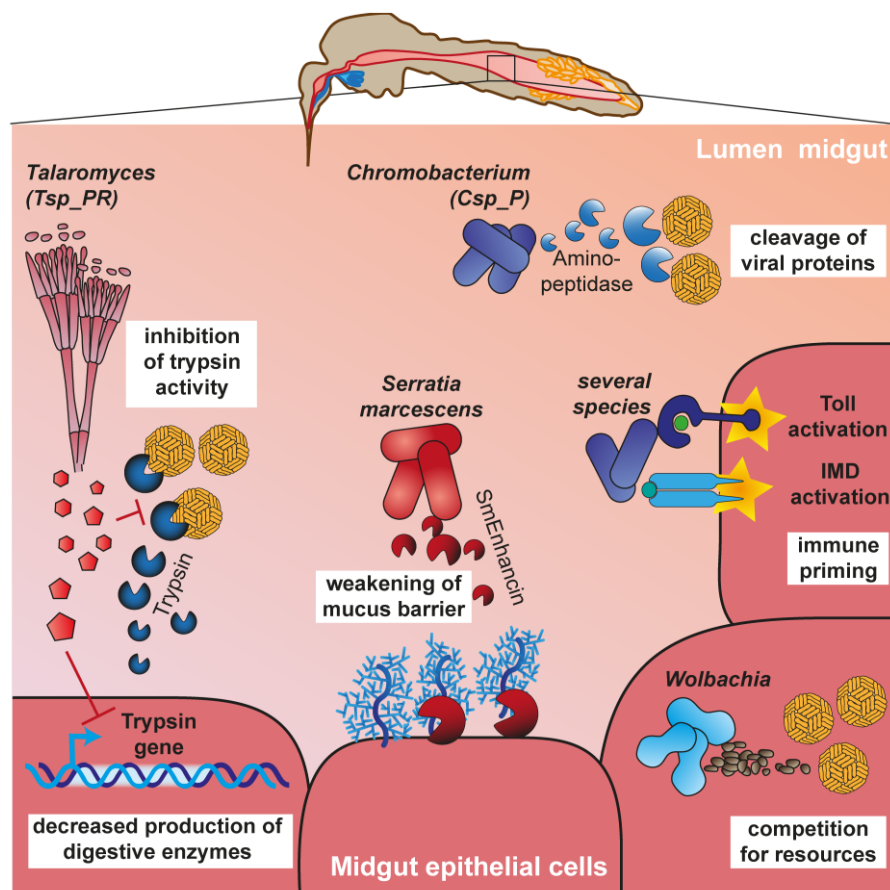


FIGURE LEGEND

Mechanisms of microbiota-mediated modulation of arbovirus infection in mosquitoes. Distinct bacterial or fungal species in the mosquito midgut can enhance (factors colored in red) or interfere with (factors colored in blue) arbovirus infections. *Talaromyces* fungi favor virus infectivity by suppressing the expression and activity of digestive enzymes. *Serratia marcescens* bacteria secrete SmEnhancin proteases that cleave membrane-bound mucins (Wu et al., 2019). *Chromobacterium* Csp_P secretes aminopeptidases that degrade viral envelope proteins. Immune priming by several microbes or competition for resources may indirectly interfere with virus replication. Note that the intracellular bacterium *Wolbachia* does not naturally occur in *Aedes aegypti* mosquitoes, but its introduction reduces replication of multiple arboviruses.

ACKNOWLEDGMENTS

We thank Joep Joosten for critical reading of the manuscript. This work is financially supported by a Consolidator Grant from the European Research Council under the European Union's Seventh Framework Programme (grant number ERC CoG 615680) and a VICI grant from the Netherlands Organization for Scientific Research (grant number 016.VICI.170.090) to R.P.v.R.

REFERENCES

- Anglero-Rodriguez, Y.I., Talyuli, O.A.C., Blumberg, B.J., Kang, S., Demby, C., Shields, A., Carlson, J., Jupatanakul, N., and Dimopoulos, G. (2017). An *Aedes aegypti*-associated fungus increases susceptibility to dengue virus by modulating gut trypsin activity. *Elife* 6, e28844.
- Barletta, A.B.F., Nascimento-Silva, M.C.L., Talyuli, O.A.C., Oliveira, J.H.M., Pereira, L.O.R., Oliveira, P.L., and Sorgine, M.H.F. (2017). Microbiota activates IMD pathway and limits Sindbis infection in *Aedes aegypti*. *Parasite Vector* 10, 103.
- Dennison, N.J., Jupatanakul, N., and Dimopoulos, G. (2014). The mosquito microbiota influences vector competence for human pathogens. *Curr Opin Insect Sci* 3, 6-13.
- Kato, N., Mueller, C.R., Fuchs, J.F., Mcelroy, K., Wessely, V., Higgs, S., and Christensen, B.M. (2008). Evaluation of the Function of a Type I Peritrophic Matrix as a Physical Barrier for Midgut Epithelium Invasion by Mosquito-Borne Pathogens in *Aedes aegypti*. *Vector-Borne Zoonot* 8, 701-712.
- Moreira, L.A., Iturbe-Ormaetxe, I., Jeffery, J.A., Lu, G.J., Pyke, A.T., Hedges, L.M., Rocha, B.C., Hall-Mendelin, S., Day, A., Riegler, M., *et al.* (2009). A *Wolbachia* Symbiont in *Aedes aegypti* Limits Infection with Dengue, Chikungunya, and Plasmodium. *Cell* 139, 1268-1278.
- Palmer, W.H., Varghese, F.S., and van Rij, R.P. (2018). Natural Variation in Resistance to Virus Infection in Dipteran Insects. *Viruses* 10, E118.
- Saraiva, R.G., Fang, J.R., Kang, S., Anglero-Rodriguez, Y.I., Dong, Y.M., and Dimopoulos, G. (2018). Aminopeptidase secreted by *Chromobacterium* sp. Panama inhibits dengue virus infection by degrading the E protein. *Plos Neglect Trop D* 12, e0006443.
- Wang, P., and Granados, R.R. (1997). An intestinal mucin is the target substrate for a baculovirus enhancin. *P Natl Acad Sci USA* 94, 6977-6982.
- Wu, P., Sun, P., Nie, K., Zhu, Y., Shi, M., Xiao, X., Liu, H., Liu, Q., Zhao, T., Chen, X., *et al.* (2019). A gut commensal bacterium promotes mosquito permissiveness to arboviruses. *Cell Host & Microbe*.
- Xi, Z.Y., Ramirez, J.L., and Dimopoulos, G. (2008). The *Aedes aegypti* Toll pathway controls dengue virus infection. *Plos Pathog* 4, e1000098.